Effect of AP102, a subtype 2 and 5 specific somatostatin analog, on glucose metabolism in rats

Tarasco, Erika ; Seebeck, Petra ; Pfundstein, Svende ; Daly, Adrian F ; Eugster, Philippe J ; Harris, Alan G ; Grouzmann, Eric ; Lutz, Thomas A ; Boyle, Christina N

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Effect of AP102, a subtype 2 and 5 specific somatostatin analog, on glucose metabolism in rats

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Abstract

Somatostatin analogs (SSA) are widely used to treat conditions associated with hormonal hypersecretion such as acromegaly and metastatic neuroendocrine tumors. First generation SSA, such as, octreotide and lanreotide have high affinity for somatostatin receptor (SSTR) subtype 2 (SSTR2), but have incomplete efficacy in many patients. Pasireotide targets multiple SSTRs, having the highest affinity for SSTR5, but causes hyperglycemia and diabetes mellitus in preclinical and clinical studies. AP102 is a new SSA with high affinity at both SSTR2 and SSTR5. We aimed to characterize the effects of AP102 versus pasireotide on random and dynamic glucose levels, glucoregulatory hormone concentrations and growth axis measures in healthy Sprague-Dawley rats. Three doses of each compound were evaluated under acute conditions (1, 10 and 30 µg/kg s.c.), and two doses during a chronic (4-week) infusion (3 and 10 µg/kg/h s.c.). Neither acute nor chronic AP102 administration altered blood glucose concentrations or dynamic responses following an intraperitoneal glucose tolerance test (ipGTT). In contrast, acute and chronic pasireotide dosing increased random and post-ipGTT blood glucose measures, compared to vehicle-treated controls. Both AP102 and pasireotide acutely suppressed growth hormone (GH) levels, although insulin-like growth factor-1 (IGF-1) and somatic growth was suppressed to a greater extent with pasireotide. AP102 is a new dual SSTR2/SSTR5-specific SSA that acutely reduces GH but does not cause hyperglycemia during acute or chronic administration in a healthy rat model. Further studies in diabetic animals and in humans are necessary to determine the potential utility of AP102 in the clinical setting.

Introduction

Acromegaly is a rare, chronic disorder characterized by excess secretion of growth hormone (GH) and insulin-like growth factor 1 (IGF-1), usually due to a somatotrope cell tumor of the pituitary gland [1]. The main medical treatment option is administration of somatostatin analogs (SSAs; also known as somatostatin receptor ligands), which inhibit GH and IGF-1 secretion and can shrink pituitary tumor size [2]. SSAs are also used in the treatment of metastatic neuroendocrine tumors (NET) in order to control signs and symptoms associated with tumoral secretion of neurotransmitters and hormones. The established SSAs, octreotide and lanreotide, mediate their actions via somatostatin receptor subtype 2 (SSTR2). However, these SSTR2-specific compounds have incomplete efficacy in the clinical management of acromegaly and often patients do not achieve the high levels of strict hormonal control reported in randomized clinical trials [3,4]. The newer SSA, pasireotide, has high affinity for multiple SSTRs, particularly SSTR5 and is approved for the treatment of Cushing's disease (via SSTR5-mediated ACTH inhibition), in addition to acromegaly [5-7]. Apart from the wider SSTR binding profile, the interactions of pasireotide with SSTR2 differs from that of octreotide due to specific patterns of phosphorylation and internalization [8-10]. Expanding the SSTR binding profile for SSA beyond SSTR2 alone has clinical benefit. For instance, in acromegaly patients that were insufficiently controlled with octreotide, switching to pasireotide led to improved GH and IGF-1 control in 17.3% of cases [11]. However, the SSTR binding and interaction profile of pasireotide also leads to undesirable effects on glucose metabolism that are not seen with SSTR2-specific compounds.

In the rat, chronic administration of pasireotide but not octreotide led to increased glucose levels; this was associated with decreased insulin in both groups as compared with controls, while octreotide also blunted glucagon secretion [12]. Following a glucose tolerance test (GTT) in healthy human volunteers, pasireotide treatment decreased insulin and incretin responses, leading to increased glycemia; this effect could be ameliorated with concomitant use of certain anti-diabetic medications [13,14]. In clinical trials of pasireotide examining both the subcutaneous and long acting repeatable (LAR) formulations, adverse events and discontinuations attributable to hyperglycaemia and diabetes mellitus occurred with greater frequency than in patients treated with octreotide/lanreotide [6,7,11,15-

17]. Use of pasireotide in acromegaly and Cushing's disease requires specific vigilance to recognize hyperglycemia and to treat new onset diabetes mellitus using anti-diabetic medication [18-20].

Given the incomplete clinical control achieved with established SSA and the issues relating to glucose metabolism with pasireotide, interest remains in new SSAs with a different profile of SSTR binding than existing compounds. AP102 is a disulfide-bridged octapeptide SSA containing synthetic iodinated amino acids (Compound 9 in [21]). Receptor binding studies have previously demonstrated that AP102 binds with subnanomolar affinity to SSTR2 and SSTR5 (IC50 : 0.63 and 0.65 nM, respectively), which were similar to the affinities obtained for native somatostatin 14 in the same experiments (IC50 : 0.4 and 0.2 nM, respectively) [21]. Further, AP102 does not bind to SSTR1 or SSTR3, which is in stark contrast to pasireotide [21,22]. AP102 also inhibited GHRH-stimulated GH release and prolactin release from rat anterior pituitary cells in vitro [21]. The IC50 levels for GH and prolactin inhibition were low (0.15 and 0.23 nM, respectively) and were similar to those obtained with somatostatin 14 (0.1 and 0.2 nM, respectively). Given this profile of high SSTR2 and SSTR5 affinity and GH/prolactin inhibition in vitro, AP102 might represent an interesting candidate for use in the clinical treatment of acromegaly or NET.

The aim of this study was to characterize the effects of AP102 on in vivo hormonal secretion and glucose metabolism following acute (single dose) and chronic (28-day) administration in Sprague Dawley rats and to compare the profile of AP102 with that of pasireotide at identical dose levels.

Methods

Animals

All experiments were performed with male Sprague Dawley rats (Janvier Labs, Le Genest-St-Isle, France). Rats were approximately 5 weeks old on arrival at the study center, which corresponds to a period of rapid somatic growth in young animals. The experiments were approved by the Kantonales Veterinäramt Zurich, Switzerland. Upon arrival at the facility, rats were given at least one week to acclimate to the new environmental and housing conditions.

Compounds

AP102 was synthesized as acetate salt by Polypeptide (San Diego, CA, USA). Commercially available pasireotide (Signifor; Novartis Pharma Schweiz) was used. AP102 and pasireotide were reconstituted and diluted in sterile 0.9% NaCl, which was also used as vehicle control, and 500-µl aliquots of 0.03 µg/ml were stored at -20 °C until use. On the day of testing, aliquots were diluted with sterile saline to the appropriate concentration, and kept on ice until used.

Single-dose subcutaneous administration study

56 male Sprague Dawley rats (150 g at arrival, approx. 5 weeks in age) were group housed (3 to 4 rats per cage; Tecniplast cage type IV S; 48 x 38 x 21 cm) under a 12/12-h light-dark cycle (lights on at 07.00 h) in a temperature-controlled environment, with ad libitum access to standard chow (D3430; Provimi-Kliba, Kaiseraugst, Switzerland) and water. Rats of this age are in an active growth phase; therefore body weight was measured daily throughout the experiment.

When weighing approximately 300 g, rats received chronic catheters into the carotid artery fitted to a vascular access button (VAB95BS; Instech Laboratories, Inc) exteriorized between the scapulae and covered with a protective cap to facilitate group housing. Rats were initially sedated with 4-5%

isoflurane (O_2 flow rate at 0.8 l/min), and maintained at 2-3% isoflurane for the remainder of the surgery. Rats were treated postoperatively with fluids (5 ml sterile Ringer-Lactate SC), antibiotics (Enrofloxacin; 5.7 mg/kg SC), and analgesics (Flunixin; 1 mg/kg SC). Following catheter implantation, infusion lines were filled with a lock solution (100 U/ml heparin in 50% glycerol), and were not manipulated until the day of testing, unless there was blood visible in the line.

One week after catheterization, rats with patent catheters (75 % of operated rats) were assigned randomly across home cages to treatment groups and injected with AP102 (1, 10, and 30 µg/kg), pasireotide (1, 10, and 30 µg/kg), or vehicle subcutaneously (n=6 analyzed for each group), beginning 90 min after light onset (08:30h). Blood samples (300 µl) were withdrawn from the carotid catheter before treatment (~08:00h), and at 0.25, 0.5, 1, 3, and 6 h post-administration. Blood glucose was measured directly from whole blood using a Nova StatStrip glucometer (DSI; St. Paul MN, USA). The remaining blood sample was collected in Na-EDTA-coated tubes containing a general protease inhibitor cocktail (P2714; Sigma; 30 µl of 1:10 diluted stock solution/1 ml blood) and a DPP-4 inhibitor (DPP-4-010; Millipore; 10 µl/1 ml blood). Samples were kept at 4 °C until spun for 10 min at 1000 x g (4 °C), and then plasma was immediately aliquoted into separate tubes and stored at -80 °C until assayed for insulin, glucagon and GLP-1 in duplicate (K15159C-1; Mesoscale Mouse/Rat GLP-1 (7-36) amide, Insulin, Glucagon custom kit), and GH in triplicate (EZRMGH-45K; Merck-Millipore Rat/Mouse Growth Hormone ELISA Kit).

Chronic 28- day continuous subcutaneous infusion study

Animals: 44 male Sprague Dawley rats (180 g at arrival) were group housed as described before. Body weight was measured at least three times per week. On day 0, under isoflurane anesthesia and prior to minipump implantation, the nose-to-anus and the tail length were measured. Body length measurements were taken again on days 14 and 28. Femur length was also recorded at necropsy on day 28.

Mini-pump Preparation and Implantation: Mini-pumps for sub-cutaneous administration (Alzet model 2ML2) were filled with the AP102, pasireotide or vehicle one day prior to implantation, and then left to equilibrate at 37 °C in sterile saline so that pumps were primed when implanted. The first day of implantation was designated as day 0. Under sterile conditions, a small incision was made between the scapulae and the mini-pump was subcutaneously implanted. Rats were initially sedated with 4-5% isoflurane (O_2 flow rate at 0.8 l/min), and maintained at 2-3% isoflurane for the remainder of the surgery. Immediately following surgery and 1 day post-op (after the day 1 blood sample), rats received SC injection of 5 ml Ringer-Lactate, antibiotics (Enrofloxacin; 5.7 mg/kg SC injected into a subcutaneous NaCl depot), and analgesics (Flunixin; 1 mg/kg SC). After 2 weeks of infusion, the minipumps were removed under isoflurane anesthesia, and replaced with a second 2ML2 minipump containing the same infusate for the second 2-week period. Treatment and groups were as follows: vehicle (n=8); AP102 3 µg/kg/h (n=9); AP102 10 µg/kg/h (n=9); pasireotide 3 µg/kg/h (n=8); pasireotide 10 µg/kg/h (n=8). Rats were semi-randomly assigned to treatment groups, ensuring no difference in day 0 body weight across groups. Acute and chronic doses where chosen based on previously published studies investigating the effects of pasireotide on glucose homeostasis in rats [12].

Blood samples (500 µl) were collected from the sublingual vein immediately before mini-pump implantation on day 0, and on days 1, 7, 14, and 21 after the start of treatment. Blood samples were collected between 10 :00 and 12 :00 each day, with the exception of the Day 0 sample, which was collected immediately before minipump implantation (08:00). Immediately after being briefly anesthetized by inhalation of 5% isoflurane, a sublingual vein was punctured with a 20 G needle, and blood was collected in Na-EDTA-coated tubes containing protease inhibitors, as described above. Samples were kept at 4 °C until spun for 10 min at 1000 x g (4 °C), and then plasma was immediately aliquoted into separate tubes and stored at -80 °C until assayed. On day 28, rats were deeply anesthetized with isoflurane, the heart was exposed, and a terminal blood sample of 2 to 3 ml was collected via heart puncture, and plasma was extracted and stored as above. Glucose, insulin,

glucagon and GLP-1 were measured as before. IGF-1 was measured in triplicate using the Rat/Mouse IGF-1 ELISA kit (AC-18F1; Immunodiagnostics Systems Ltd).

On day 21 of treatment, rats were briefly anesthetized by inhalation of 5% isoflurane, placed in a supine position and the tongue extended from the mouth using a cotton-tipped applicator. One of the sublingual veins was punctured with a 20 G needle, and 500 µl blood was collected in Na-EDTA-coated tubes and treated with inhibitors, as described above. This sample was used for day 21 baseline analyses and for pre-glucose comparison; Blood glucose was measured directly from whole blood using Nova StatStrip glucometer (Data Sciences International). Before recovery from isoflurane, rats were injected i.p. with 1.5 g/kg glucose (50% glucose solution). Rats were then allowed to recover from anesthesia. Thirty minutes later, rats were again anesthetized and a second sublingual blood sample of 300 µl was collected as described above. A full glucose tolerance test time-course was not performed due to limitations in the volume of blood safely collected from the rats. Only rats demonstrating a rise in blood glucose above 200 mg/dL 15 min after glucose injection were included in the analysis. Resulting group sizes were: Vehicle (n=4); AP102 3 µg/kg/h (n=6); AP102 10 µg/kg/h (n=6); pasireotide 3 µg/kg/h (n=6); pasireotide 10 µg/kg/h (n=6).

Determination of AP102 stability in the experimental conditions

Three mini-pumps were prepared under identical conditions as described above, but were not implanted in rats. AP102 solutions at 0.19 and 0.64 mg/mL (equivalent to 3 µg/kg/h and 10 µg/kg/h AP102 infusate solutions) or saline were placed in the mini-pumps, infused during 13 days at 37°C and collected in Eppendorf tubes every day. The peptide content was quantified by liquid chromatography–mass spectrometry (LC-MS/MS) in these samples, in residual solution from the mini-pumps, and in stock and working solutions described in acute and chronic experiments to assess its stability and the absence of adsorption on surfaces. All samples were 60-fold diluted in saline before analysis.

Statistical Analysis

Statistical analysis was done using Prism 7 (Graph Pad Software, La Jolla, CA, USA). One-way and two-way repeated measures ANOVA analyses were followed by Bonferroni adjustment for multiple comparisons, when appropriate. Total area under the curve (AUC) was calculated using the trapezoidal rule. A P-value of < 0.05 was considered statistically significant for all experiments.

Results

The acute study was performed to determine the effect of single doses of AP102 and pasireotide on blood glucose and GH levels. Six hours after acute treatment, we observed significant reductions in circulating GH in rats treated with pasireotide (10 and 30 μg/kg doses [$p < 0.001$ for both]) and AP102 (1 μg/kg [$p < 0.05$] and 30 μg/kg doses [$p < 0.001$]), as compared to vehicle-treated rats (Figure 1A). The corresponding AUC analysis revealed that 10 and 30 μg/kg pasireotide ($p < 0.01$ for both) and 30 μg/kg AP102 ($p < 0.05$) suppressed GH levels compared to vehicle (Figure 1A inset). Acute administration of AP102 had no effect on glucose levels (Figure 1B). Pasireotide treatment led to increased plasma glucose in all three dose-groups during the first hour and remained significantly elevated in the highest dose group at 6 hours. We were unable to detect differences in plasma insulin, glucagon, or GLP-1 following acute administration of AP102 and pasireotide due to high variability in the samples (data not shown). We suspect the high variability resulted from not fasting the rats prior to the acute injection; thus we could not control for the time since the last meal, which can affect the levels of these metabolic hormones.

Chronic AP102 treatment transiently decreased blood glucose levels as compared to vehicle (Figure 2A). The effect was significant on the first day of infusion in both the 3 μg/kg/h ($p<0.05$) and 10 μg/kg/h ($p<0.001$) groups. This remained significant in the 10 μg/kg/h group until day 7 of infusion ($p<0.01$); AUC showed that both AP102 doses lowered glucose levels compared to vehicle ($p<0.001$ for both; Figure 2A inset). Pasireotide led to an initial, non-significant rise in plasma glucose, and glucose levels remained at or above those of vehicle for the whole study duration. Insulin levels were suppressed in both AP102 and pasireotide treated groups as compared with vehicle treated (Figure 2B AUC inset; $p<0.001$ for all group). These differences did not reach statistical significance at individual time points, likely due to large variability in the control group. Similarly, glucagon levels were similar in all study groups up to day 21, but were significantly lower in the AP102 treated groups ($p<0.01$ for both) than in the vehicle or pasireotide treated groups on day 28 (Figure 2C). GLP-1 levels decreased in the pasireotide treated groups immediately after infusion began, being significantly decreased versus vehicle in the 3 μg/kg/h pasireotide group on Day 1 (Figure 2D).

Thereafter GLP-1 levels remained numerically lower than vehicle throughout the study and lower overall according to AUC ($p<0.001$ for both; Figure 2D inset), whereas in AP102 treated rats GLP-1 levels were generally at or above those seen with vehicle and pasireotide.

On day 21 of the chronic treatment period, rats underwent an ipGTT. AP102 had no impact on plasma glucose levels following ipGTT. Glucose was significantly increased as compared to vehicle in the 3 μg/kg/h pasireotide group at 60 min ($p < 0.05$; Figure 3A). The rise in insulin 30 minutes after glucose administration was significantly lower in the pasireotide-treated groups than vehicle treated controls ($p<0.01$; Figure 3B and inset). AP102 did not differ significantly from vehicle in terms of insulin levels post ipGTT (Figure 3B), though AUC was significantly lower in the 3 μg/kg/h AP102 group compared to vehicle ($p<0.05$; Figure 3B inset). During the ipGTT, glucagon levels in the AP102 groups remained below the lower limit of detection at 30 minutes, whereas pasireotide groups demonstrated numerically higher glucagon levels than controls (Figure 3C). At baseline before the ipGTT, AP102 treated groups had numerically higher GLP-1 levels, which remained higher than vehicle and pasireotide groups at the 30 minute time point (Figure 3D), but did not reach statistical significance.

Chronic infusion of AP102 significantly reduced IGF-1 on the first full day after the start of the infusion ($P<0.001$ in both dose groups); thereafter IGF-1 remained 15-20% lower than the vehicle group through to the end of the study; though not statistically significant at individual time points, AUC was significantly lower in both the 3 μg/kg/h ($p < 0.01$) and 10 μg/kg/h ($p < 0.001$) AP102 groups compared to vehicle (Figure 4). Both doses of pasireotide suppressed IGF-1 compared with vehicle across the 28-day period ($p < 0.001$), and evident from the AUC analysis ($p < 0.001$). Rats treated with both doses of pasireotide and the 10 μg/kg/h dose of AP102 gained significantly less body weight than vehicle-treated controls (p-values provided in Figure 5A). In the pasireotide groups, decreased body length ($p < 0.001$ for both doses on days 14 and 28), tail length ($p < 0.001$ for 10 μg/kg/h group on both days; $p < 0.05$ on day 14 and $p < 0.01$ on day 28 for 3 μg/kg/h group), and femur length ($p < 0.001$ for both doses on day 28) were seen. With AP102 treatment, only tail length

was decreased by the higher dose at the end of the study (day 28 in the 10 µg/kg/h group; Figures 5B-5D).

LC-MS/MS analyses showed that the stock and working solutions, the residual solution in the mini-pumps after 13 days and the samples collected from the mini-pumps after one and 13 days contained the expected amounts of peptide. These results show that AP102 was stable in the mini-pumps delivering the peptide to the rats for 13 days at 37°C. Moreover, AP102 was neither adsorbed on the surface of the mini-pumps nor on the containers used for the preparation of stock and working solutions (data not shown).

Discussion

This study aimed to characterize the effects of acute and chronic treatment of a new dual SSTR2/SSTR5 specific SSA, AP102 on glucose metabolism, hormonal measures, and growth in healthy growing rats. Following acute and chronic administration, in vivo AP102 did not lead to hyperglycemia or impaired glucose tolerance in this model. These data indicate that a low or sub-nanomolar binding affinity at SSTR2 and SSTR5 is not necessarily associated with an increased risk of hyperglycemia.

Our acute study clearly showed that AP102 does not induce hyperglycemia in rats, which contrasts the well-documented hyperglycemic effect of pasireotide in rats and humans [12,13] that we also reproduced here. The high variability in the concentrations of glucoregulatory hormones that we observed in the acute study did not allow us to draw conclusions about the underlying mechanisms following a single treatment. The results of the chronic study, however, provide additional insight, suggesting that AP102 likely influences glycemia by a more balanced suppression of insulin and glucagon compared to pasireotide.

In our chronic study, AP102 was also associated with lower glucose concentrations as compared with vehicle and pasireotide treated groups, while insulin was reduced to a similar degree as with pasireotide. In an earlier experiment, in which healthy rats were infused with pasireotide at the same doses we administered for 14 days duration, pasireotide was associated with increased plasma glucose levels at days 1 and 7 versus a vehicle-treated group [12]. In that study, the comparator was the SSTR2-specific compound octreotide, which was associated with a tendency to lower blood glucose during continuous infusion. While both octreotide and pasireotide reduced plasma insulin concentrations, octreotide was associated with a concomitant reduction in glucagon that did not occur with pasireotide. Like octreotide, AP102 lowered glucagon levels, most notably at the end of the 28-day infusion period. Suppression of glucagon by AP102 appears to offset the inhibitory effect on insulin, resulting in a net glucose-neutral effect during chronic administration and following the ipGTT. The results of the ipGTT on day 21 further support the interpretation that chronic AP102

modifies glucose-stimulated hormone release in different and less diabetogenic manner than pasireotide. Specifically, glucagon was completely undetectable in the AP102 groups 30 minutes after glucose challenge, and only the pasireotide-treated groups had significant blunting of glucose-simulated insulin levels. Findings from the chronic and ipGTT studies also lead us to hypothesize that GLP-1 plays a secondary role in counteracting hyperglycemia after AP102 treatment. We observed lower GLP-1 levels in the groups treated chronically with pasireotide, while chronic AP102 treatment resulted in GLP-1 levels numerically higher than the vehicle-treated group. GLP-1 levels in the AP102 groups were also numerically higher than the vehicle and pasireotide groups both at the beginning of the ipGTT and 30 minutes after glucose stimulation. Human data showing that pasireotide can suppress glucose-stimulated GLP-1 release suggest that the hyperglycemic effects of pasireotide are explained by a combination of insulin and incretin inhibition [13]. While the incretin effect of GLP-1, whereby GLP-1 increases insulin secretion, is well established, it is also known that GLP-1 can enhance insulin activity and improve glucose tolerance independently of modifying insulin release [23-25]. Thus the increased, or at least non-suppressed, GLP-1 levels following AP102 treatment might help to counter the reduced insulin levels by enhancing insulin action and thereby contributing to the net lowering of glucose. Taken together these results suggest that AP102 has a neutral effect on glucose via a balanced inhibition of insulin and glucagon release, and potential enhancement of insulin action resulting from uninhibited GLP-1 levels.

The other main aim of the study was to assess the effect of AP102 on GH and IGF-1 secretion. Six hours after a single treatment, the highest dose of AP102 reduced GH levels compared to vehicle-treated rats, and the effect of AP102 was comparable to that produced by the two highest doses of pasireotide. The high inter-animal variability in GH levels across treatment groups and time points likely results from the short half-life and pulsatile pattern of release of GH, substantiating claims that the direct measurement of GH in plasma is challenging and can lack utility when assessing the efficacy of SSA-based treatments [26]. IGF-1 levels, which provide an indirect readout of GH activity, were measured in the chronic study to circumvent these limitations. AP102 strongly suppressed IGF-1 after one day of continuous infusion, and then maintained IGF-1 levels at 15-20%

of baseline, while pasireotide lowered IGF-1 to approximately 50% of baseline over the 28-day study. Normal growth was also significantly reduced by pasireotide versus controls in terms of femur length, body weight, and body and tail length. Similar to its effect on IGF-1, AP102 led to numerically lower growth measures than controls, but this was only significant for inhibition of tail length at the end of the 28-day study. The effects of AP102 and pasireotide on physiological GH-IGF-1 axis function and somatic growth clearly differ. This mirrors what has been established regarding octreotide. Octreotide has only a transient inhibitory effect on physiological secretion of GH and IGF-1 in the rat. This was first established by Lamberts et al in 1987, who demonstrated that daily administration of octreotide in healthy rats initially led to GH inhibition for up to 6 days [27]. Thereafter, the effect of octreotide was desensitized and at day 15 GH levels were identical in octreotide-treated and control rats. Schmid & Brueggen also found a similar desensitization of the chronic effect of octreotide in healthy rats, which contrasted with the significant inhibition of physiological IGF-1 secretion and growth with pasireotide [12]. Pasireotide also inhibits GH/IGF-1 secretion in patients with normal GH axis, as has been shown in Cushing's disease [28]. These findings highlight a limitation of the normal rat model in comparing the potential hormonal profile of SSA in the pathological setting of acromegaly in humans. Despite the transient effect on hormonal secretion in the rat, octreotide effectively controls pituitary tumor related GH and IGF-1 hypersecretion in acromegaly during chronic treatment for years without desensitization occurring.

The explanation of why AP102 and pasireotide produced very different patterns of effects on glucose metabolism, despite both having high affinities for SSTR2 and SSTR5, requires further investigation. There are, however, a number of factors that are likely to play a role in this difference. Pasireotide binds with high affinity to other somatostatin receptors apart from SSTR2 and SSTR5, such as SSRT3 and SSTR1, whereas AP102 is limited to SSTR2 and SSTR5 only. Effects at these other receptors might play a role in pasireotide-related glucose excursions. For instance, SSTR3 is highly expressed in the pancreatic islets of humans and rodents and recently SSTR3 antagonists have been developed for the treatment of type 2 diabetes [29]. Pasireotide has also been shown to differ significantly from other somatostatin analogs in terms of its phosphorylation profile at certain SSTRs [9,30]. It is also

important to note that SSA-binding affinities do not directly predict the functionality at the specific SSTRs [31]. For example, pasireotide was shown to activate a different pattern of signal transduction than octreotide upon binding to SSTR2 [32]. The interactions between AP102 and SSTR2 and SSTR5 receptors have not yet been studied in this manner, but could contribute to the differences from pasireotide in SSTR-driven effects on glucose metabolism that we report.

In conclusion, administration of AP102 was not associated with impairment of glucose control in a healthy rat model, which suggests that balanced, high affinity binding to SSTR2 and SSTR5 can be achieved without diabetogenic effects. The risk of impaired glucose control in humans with acromegaly treated with pasireotide is higher in those with elevated pre-treatment blood glucose levels. Therefore, further comparative studies using AP102 in animal models of type 2 diabetes could help to clarify the potential utility of AP102 in clinical practice, especially for patients in whom diabetes is a highly undesirable complication of treatment.

Declaration of interest

A.F.D., A.G.H. and E.G. have consulting or stock holding relationships with Amryt Pharmaceuticals. E.T., P.S., S.P., P.J.E., T.A.L., and C.N.B. have nothing to disclose.

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Author Contributions

Conception and design of the work: A.F.D., A.G.H., E.G., T.A.L, C.N.B.

Data collection: E.T., P.S., S.P., C.N.B; Data analysis and interpretation: E.T., A.F.D, P.J.E., A.G.H, E.G, T.A.L., C.N.B; Drafting the article: T.A.L, C.N.B.; Critical revision of the article: E.T., A.F.D, P.J.E, E.G., T.A.L, C.N.B; Final approval of the version to be published: E.T., P.S., S.P., A.F.D, A.G.H., P.J.E., E.G, T.A.L., C.N.B

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Figure Legends

Figure 1: Mean (± SEM) plasma growth hormone (A) and blood glucose (B) levels measured before and 0.25, 0.5, 1, 3, and 6 hours after SC treatment with vehicle (sterile saline), pasireotide (1, 10, or 30 μg/kg) or AP102 (1, 10, or 30 μg/kg) in male rats. Insets show mean (± SEM) area under the curve (AUC) for growth hormone (A) and glucose levels (B) over the six-hour test period. Symbols denote significant differences from vehicle treatment: pasireotide 30 μg/kg: **P≤0.01, ***P≤0.001; pasireotide 10 μg/kg: +P≤0.05, ++P≤0.01, +++P≤0.001; pasireotide 1 μg/kg: °P≤0.05, °°°P≤0.001; AP102 30 μg/kg: ^P≤0.05, ^^^P≤0.001; AP102 1 μg/kg: †P≤0.05

Figure 2: Mean (± SEM) blood glucose (A), plasma insulin (B), plasma glucagon (C), and plasma GLP-1 (D) levels measured before and following 1, 7, 14, 21, and 28 days of chronic SC treatment with vehicle (sterile saline), pasireotide (3 or 10 μg/kg/h) or AP102 (3 or 10 μg/kg/h) in male rats. Insets show mean (± SEM) area under the curve (AUC) for glucose (A), insulin (B), glucagon (C), and GLP-1 levels (D) during the 28-day treatment period. Symbols denote significant differences from vehicle treatment: pasireotide 10 μg/kg/h: ***P≤0.001; pasireotide 3 μg/kg/h: +P≤0.05, ++P≤0.01, +++P≤0.001; AP102 10 μg/kg/h: °°P≤0.01; °°°P≤0.001; AP102 3 μg/kg/h: ^P≤0.05, ^^P≤0.01, ^^^P≤0.001

Figure 3: Mean (± SEM) blood glucose (A) and glucose-stimulated plasma levels of insulin (B), glucagon (C), and GLP-1 (D) following IP glucose tolerance test in male rats chronically treated for 21 days with vehicle (sterile saline), pasireotide (3 or 10 μg/kg/h) or AP102 (3 or 10 μg/kg/h). Insets show mean (± SEM) area under the curve (AUC) for glucose (A), insulin (B), glucagon (C), and GLP-1 levels (D) in response to the glucose challenge. Symbol denotes significant differences from vehicle treatment at individual time points: pasireotide 10 μg/kg/h: **P≤0.01; pasireotide 3 μg/kg/h: +P≤0.05, ++P≤0.01; AP102 3 μg/kg/h: ^P≤0.05

Figure 4: Mean (± SEM) plasma IGF-1 levels measured before and following 1, 7, 14, 21, and 28 days of chronic SC treatment with vehicle (sterile saline), pasireotide (3 or 10 μg/kg/h) or AP102 (3 or 10 μg/kg/h) in male rats. Insets show mean (± SEM) area under the curve (AUC) for IGF-1 during the 28-day treatment period. Symbols denote significant differences from vehicle treatment at individual time points: pasireotide 10 μg/kg/h: ***P≤0.001; pasireotide 3 μg/kg/h: +++P≤0.001; AP102 10 μg/kg/h: °°°P≤0.001; AP102 3 μg/kg/h: ^^P≤0.01, ^^^P≤0.001

Figure 5: Mean (± SEM) body weight (A) measured daily before and during 28-day chronic SC treatment with vehicle (sterile saline), pasireotide (3 or 10 μg/kg/h) or AP102 (3 or 10 μg/kg/h) in male rats. Mean (± SEM) femur (B), nose-to-anus (C), and tail (D) length were measured following 14 and/or 28 days of chronic treatment. Symbols denote significant differences from vehicle treatment: pasireotide 10 μg/kg/h: **P≤0.01; ***P≤0.001; pasireotide 3 μg/kg/h: +P≤0.05; ++P≤0.01; +++P≤0.001; AP102 10 μg/kg/h: °°P≤0.01; °°°P≤0.001

Figure 1

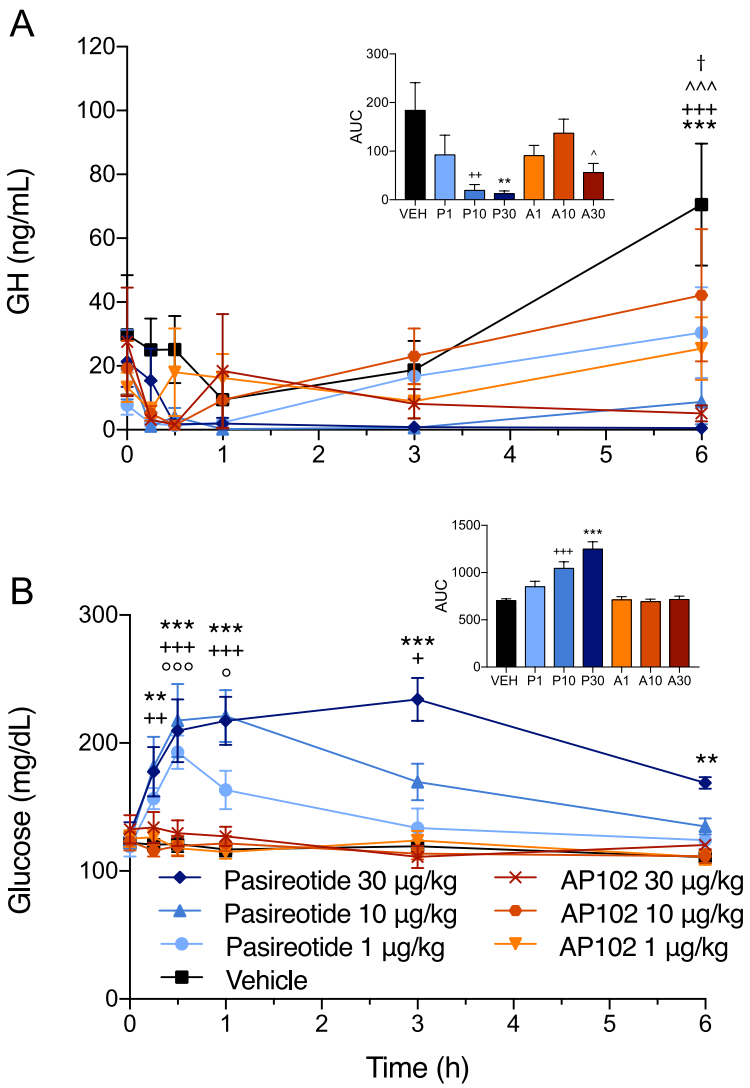

A
GH (ng/mL)
AUC
VEH P1 P10 P30 A1 A10 A30
B
Glucose (mg/dL)
Time (h)
Pasireotide 30 µg/kg
Pasireotide 10 µg/kg
Pasireotide 1 µg/kg
Vehicle
AP102 30 µg/kg
AP102 10 µg/kg
AP102 1 µg/kg

Figure 2

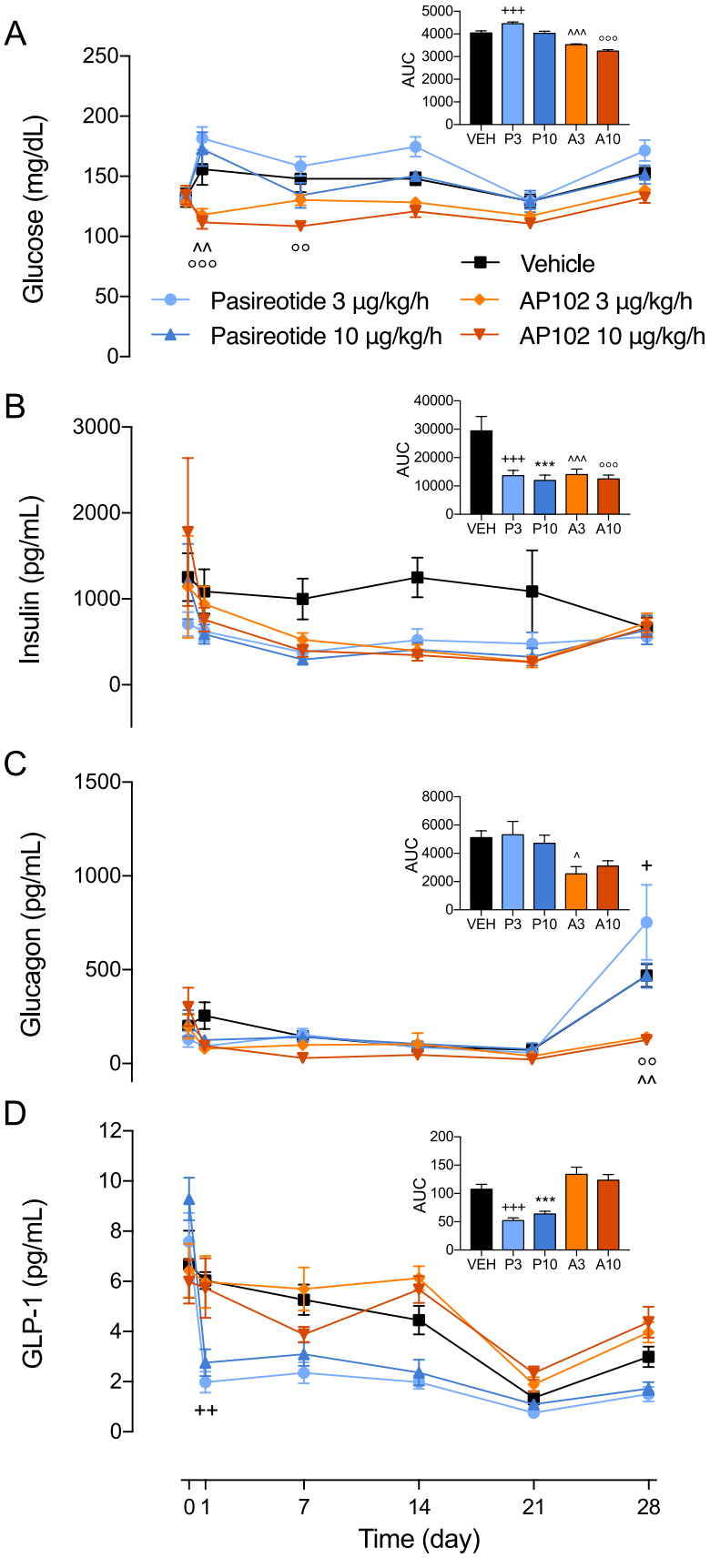

A
Glucose (mg/dL)
250
200
150
100
50
0
AUC
5000
4000
3000
2000
1000
0
VEH P3 P10 A3 A10
+++
^^^
ooo
^^
ooo
oo
Vehicle
Pasireotide 3 µg/kg/h
AP102 3 µg/kg/h
Pasireotide 10 µg/kg/h
AP102 10 µg/kg/h
B
Insulin (pg/mL)
3000
2000
1000
0
AUC
40000
30000
20000
10000
0
VEH P3 P10 A3 A10
+++
***
^^^
ooo
C
Glucagon (pg/mL)
1500
1000
500
0
AUC
8000
6000
4000
2000
0
VEH P3 P10 A3 A10
^
+
oo
^^
D
GLP-1 (pg/mL)
12
10
8
6
4
2
0
AUC
200
150
100
50
0
VEH P3 P10 A3 A10
+++
***
++
0 1
7
14
21
28
Time (day)

Figure 3

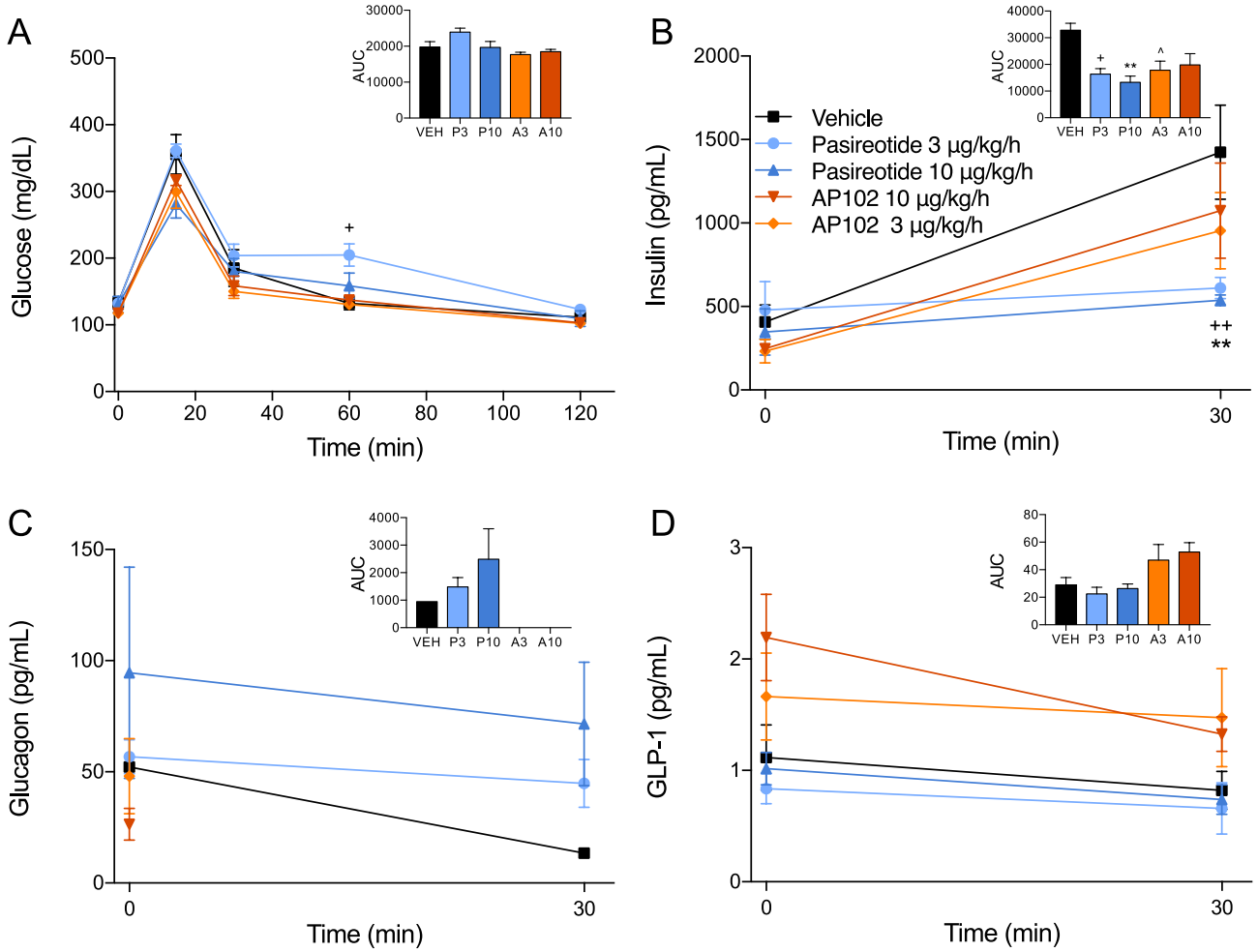

A
Glucose (mg/dL)
Time (min)
AUC
VEH P3 P10 A3 A10
B
Insulin (pg/mL)
Time (min)
Vehicle
Pasireotide 3 µg/kg/h
Pasireotide 10 µg/kg/h
AP102 10 µg/kg/h
AP102 3 µg/kg/h
C
Glucagon (pg/mL)
Time (min)
D
GLP-1 (pg/mL)
Time (min)

Figure 4

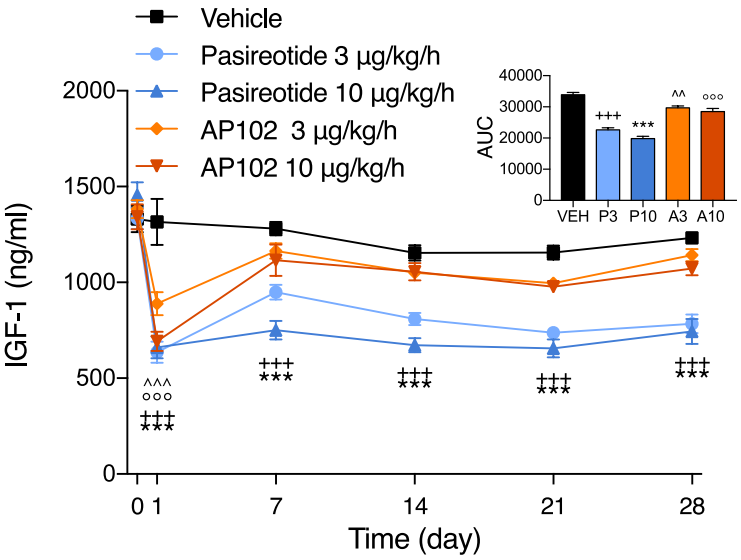
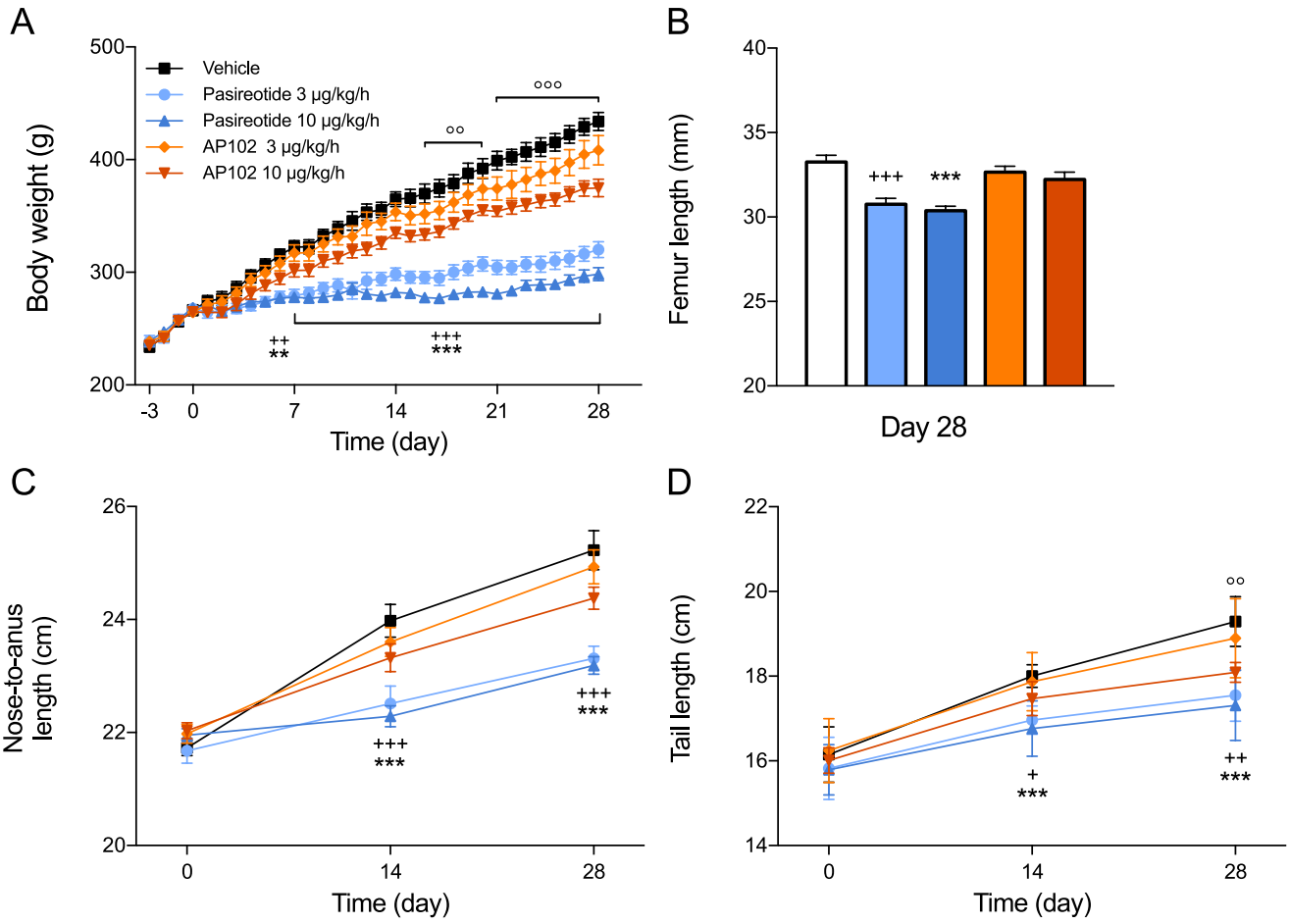

Vehicle
Pasireotide 3 µg/kg/h
Pasireotide 10 µg/kg/h
AP102 3 µg/kg/h
AP102 10 µg/kg/h
IGF-1 (ng/ml)
2000
1500
1000
500
0
0 1
7
14
21
28
Time (day)
AUC
40000
30000
20000
10000
0
VEH P3 P10 A3 A10

Figure 5


A
Body weight (g)
500
400
300
200
Vehicle
Pasireotide 3 µg/kg/h
Pasireotide 10 µg/kg/h
AP102 3 µg/kg/h
AP102 10 µg/kg/h
°°
°°°
++
**
+++
***
-3
0
7
14
21
28
Time (day)
B
Femur length (mm)
40
35
30
25
20
+++
***
Day 28
C
Nose-to-anus length (cm)
26
24
22
20
+++
***
+++
***
0
14
28
Time (day)
D
Tail length (cm)
22
20
18
16
14
°°
+
***
++
***
0
14
28
Time (day)